FERTILIZATION IN ABIES BALSAMEA

A. H. HUTCHINSON

(WITH PLATES XVI–XX AND ONE FIGURE)

A general account of fertilization and the related phenomena in this species has been given by MIYAKI (19). A number of super-numerary nuclei were noted in the micropylar end of the egg at the time of fertilization. These were generally regarded as derived from the male gametophyte, while in some cases another nucleus, that of the ventral canal cell, was added to the number. Repetition has been avoided, inasmuch as the present account is restricted to special problems, while the general account has received little attention.

The problems suggested by the regular occurrence of four nuclei in the egg cytoplasm, near the micropylar end, at the time of the 4-nucleate proembryo, and by the unusual grouping of chromosomes during the division which follows the conjugation of the egg and sperm nuclei, have led to this investigation. Special attention, therefore, will be given to the fertilization of the nucleus of the ventral canal cell and to the cytological features connected with the fertilization of the egg.

The material was collected in Ontario, Canada. I am greatly indebted to Professor W. R. SMITH for collections made at Lake Joseph. On June 25 and 26 I obtained ovules showing fertilization at N. lat. 44°, W. long. 79°12′, and on July 2 and 3 ovules showing similar stages at N. lat. 45°30′, W. long. 78°32′. The altitude in the first case was 900 feet; in the second 1800 feet.

In a former paper on the male gametophyte of *Abies* (10), the excessive number of prothallial cells has been recorded. One of these polar cells, during mitosis, is shown in fig. 1. Fig. 5, which was drawn from a pollen grain lodged in the micropyle, shows the division of the body cell nucleus, to form two male nuclei, taking place before the tube breaks through the spore coat. At this time the cytoplasm surrounding the tube nucleus is extremely vacuolate,

that of the body cell very dense. The tube nucleus begins to disintegrate before the pollen tube emerges from the exine; usually during the pollen tube stage it appears only as an irregular aggregation; seldom, if ever, does it enter the egg.

A number of gametophytes have been found similar to that shown in fig. 2. The first division has cut off a polar ("prothallial") cell which later has disintegrated; the second division has given rise to two equivalent cells; which of the two might have been regarded as prothallial, under other circumstances, is impossible to determine. Evidently there are two antheridia. The conclusions based on a study of *Picea* (9), namely, that under favorable conditions any of the cells resulting from the three primary divisions of the male gametophyte may be antheridial, are supported by the facts as found in *Abies*.

The period of time between pollination and fertilization is from four to five weeks. During the greater part of this time the pollen grains lie dormant on the nucellus, or lodged in the micropyle. The course of the pollen tube is direct, and the motion rapid. Although the rate is difficult to determine, it is believed that the passage time of the pollen tube does not exceed two days, and probably may be measured in hours.

Fertilization of the ventral canal cell

The division of the central cell to form the egg and the ventral canal cell is similar to that in *Pinus* (figs. 7, 8, 9; compare, 3, 5, 6, 19); the nuclei formed are similar in size and form. The egg nucleus at once begins to move toward the center of the egg cytoplasm (fig. 7). What the attractive force may be is unknown, but the nucleus of the ventral canal cell responds to the same force and moves in the same direction. This nucleus breaks through the cell wall and enters the cytoplasm of the egg (figs. 11, 12); here it increases in size until it reaches a length, in some cases of 80 μ, which is approximately one-half of the greatest length attained by the egg nucleus. The structure of the ventral nucleus is very similar to that of the egg, which will be described later. The latter is surrounded by a dense granular layer which is not present in the case of the former.

The fertilization of the nucleus of the ventral canal cell has been seen in several instances. One of the male nuclei fuses with the ventral nucleus (figs. 15, 17, 21); the stalk nucleus also may be in close proximity (figs. 17, 18). Sometimes two tubes enter the archegonium, in which case male nuclei from different gametophytes may fuse with the ventral nucleus and egg nucleus respectively (fig. 21). The chromatin of the fusion nucleus condenses near its center (fig. 18), and the first division takes place. Two successive divisions (figs. 16, 23, 24, 26) result in four nuclei, which as might be expected, are generally arranged in pairs (figs. 22, 25). The nuclei of this ventral proembryo range in diameter from 40 to 50 μ; those of the proembryo proper from 60 to 65 μ; otherwise the similarity is very marked (figs. 32, 53, 54).

Nuclear changes

The changes in size of the nuclei located in the egg cytoplasm are the most readily measured of all the modifications; moreover, the increase or decrease in volume will serve to indicate the extent of the qualitative changes which occur. Immediately after the division of the central cell the egg nucleus measures about 30 μ in diameter (fig. 7). At the time of fusion its length approximates 100 μ (fig. 19); during the approximation of the chromatin groups 160 μ is the maximum measurement (fig. 27), and during anaphase of the first division the diameter is again reduced to 50 or 60 μ (fig. 46). The changes in size of the daughter nuclei are less marked; the diameter varies from 20 μ at telophase (fig. 52) to 65 μ in the resting stage   It is not surprising that the egg nucleus should vary greatly in structure while increasing to 60 times its original volume, and again decreasing to one-tenth its attained volume.

During the early stages of the first division, and even before the chromatin groups have united, four differentiations of the "nuclear" material are evident. The chromatin group or groups occupy less than one-tenth of the space within the nuclear membrane (figs. 27, 28, 42). The spindle fibers are intranuclear in origin (fig. 28). Large, vacuolate, irregular, deeply staining masses are distributed throughout the whole area. The greater part of the nuclear cavity is pervaded by slender filaments, which

include small granules.   An attempt has been made to trace these structures from their origin to their fate, in order that something regarding their nature and function might be determined.   This complexity of structure is in contrast with the prophase of an ordinary mitosis, where only chromosomes, at most chromosomes and the nucleolus, are inclosed by the nuclear membrane.

The dark vacuolate masses are most conspicuous just after fusion (fig. 27).   In the early stages small refractive globules are scattered throughout the granular egg nucleus; later, these become inclosed in a gelatinous network (fig. 19), and next appear as previously described.   During the first mitosis they accumulate into several globular, vacuolate bodies (fig. 34).   The latter decrease in volume or become distributed throughout the nucleus during metaphase (fig. 46).   At anaphase the irregular masses once more become conspicuous (fig. 51).   When the daughter nuclei are formed, these bodies are not included, and soon disappear.   The fact that they are extruded would seem to indicate that they are not fundamental nuclear material.   The fact that they are stored up during the growth of the nucleus and decrease in amount during mitosis would suggest that they are simply stored food bodies.

Two successive groups of intranuclear fibers become differentiated during the processes of fusion, and the first division after conjugation.   The first is concerned with the approximation of chromosomes to be described later;   the second with the first mitosis.   While the two chromatin groups, from the egg and from the male nucleus, are still distinct, fibers which penetrate and surround these groups are organized (fig. 28).   A union of the two groups of fibers results in the formation of a single spindle made up of large complex strands (figs. 29–33).   The spindle drawn was 60 μ in length.   After the approximation of the chromosomes into pairs the spindle broadens;   the fibers become less conspicuous, and finally disappear, leaving a group of irregularly arranged chromosomes within the now much reduced nuclear space (figs. 43–45).   Meanwhile the chromosomes migrate to the center of the nucleus, and soon the second set of fibers is formed (fig. 46).   At first these are restricted to the region in which the chromosomes are situated; as the chromosomes move to the poles, the whole nucleus becomes

pervaded by fibers radiating from irregular centers (fig. 51). In the egg of *Abies* there is an excessive development of intranuclear fibers. It seems reasonable to suppose that they originate from cytoplasmic material which has entered the nucleus during its growth period, and which may be differentiated into the fibrous form under physiological conditions not yet determined.

The slender filaments pervaded by small granules are scattered throughout the greater part of the nucleus. They are most definitely organized at the time when the spindle fibers are most conspicuous (figs. 27, 33, 42). When the fibers disappear, the filaments become disorganized, resulting in a granular mass (figs. 43, 46); when the spindle fibers appear the second time, the whole nucleus becomes more or less fibrous (fig. 51); when the daughter nuclei are formed, these bodies are not included, but form a matrix for the nuclei (figs. 49, 50). We may conclude that these filaments also are cytoplasmic, resembling the spindle fibers in nature, and becoming differentiated under similar conditions.

Pairing of chromosomes in fertilization

The succession of events which occur in connection with fertilization and the first division of the zygote has been traced not only by a consideration of the stages in the approximation and redistribution of chromatin bodies, but also by tracing parallel series of changes in the size of the nucleus, in the modifications of the deeply staining food bodies, and in the formation and dissolution of spindle fibers. A study of the chromatin, involving as it does the union of the male and female elements and distribution in the daughter nuclei, is of primary importance. Emphasis has been given first to a study of the related phenomena, already described, thereby eliminating, in so far as is possible, the possibility of a misinterpretation of the order of events.

The approximation of male and female nuclei has been described for a number of conifers. In general the process as found in *Abies* agrees with that of these descriptions. A few features may be noted. No cytoplasm could be detected adhering to the male nucleus as it approached the egg nucleus. There is a great disparity in the sizes of the pairing nuclei; that of the egg, as shown in fig. 19,

is 120 μ by 80 μ, while the male nucleus is 45 μ by 15 μ. The membranes of the nuclei are resorbed at the place of contact, and the contents of the male nucleus pass into the interior of the female nucleus, thereby leaving a protuberance containing very little cytoplasm.

In *Abies* the chromatin could not be detected definitely until the formation of two groups in the micropylar end of the egg nucleus takes place. While the groups are still distinct, the individual chromosomes become separate (fig. 28), each group containing the haploid number of chromosomes. As the two spindles unite, the chromosomes become paired (figs. 21–33); at first the individuals of a pair approximate side by side (figs. 31, 32); soon they twist about one another and jointly loop into the form of a C (figs. 30, 32, 33). The chromosomes are very large, in some cases exceeding 20 μ in length. There is abundant evidence that this is a pairing, not a longitudinal splitting of chromatin elements. First, the number of pairs is haploid. This is the number which would necessarily result from a pairing of the double number of chromosomes already present; a splitting would, of course, result in $2x$ pairs. Also, the twisting of the chromosomes about one another is identical with their behavior in what is generally regarded as a pairing during the prophase of the first reduction division. Moreover, there follows a transverse segmentation. If the diploid number of chromosomes should undergo two divisions, one longitudinal and one transverse, an $8x$ number of chromosomes would necessarily result. The facts cannot be explained by the supposition that there is a longitudinal split; they are readily explained by regarding this paired appearance as a true pairing.

The segmentation.—The bending into C or V-shaped forms is followed by a segmentation of each component of the pairs; in other words, a transverse fission at the angle of the bent chromosomes (figs. 34, 40). The resulting $4x$ daughter segments are approximately 10 μ in length, or one-half the length of the pairs before and during the looping process. At first the segments remain more or less twisted about one another (figs. 34, 37, 41, 42), and for some time retain a paired relation (figs. 43, 45). They may be in the form of X's, or V's, or parallel rods. At the time when

the second set of spindle fibers begins to be differentiated, the $4x$ number of chromosomes are indiscriminately intermingled (figs. 46–48). Half the number pass to each pole to form the daughter nuclei.

The chromosome count.—Repeated chromosome counts in the sporophyte and gametophyte confirm one another in fixing the x and $2x$ numbers for *Abies balsamea* as 16 and 32. The individual chromosomes which appear during the prophase of the division in the central cell are shown in figs. 8 and 9. The count is 16. The division of the body cell in anaphase gives 39 segments. A reconstruction of parts separated by the microtome knife results in a count of 32, or 16 passing to each pole. At the time of approximation of the chromosomes in the egg, there are 16 pairs (figs. 29–33). When segmentation takes place, 32 pairs of segments are present (figs. 34–40). In the nucleus represented in figs. 43–45, there are 72 chromosome pieces; figs. 46–48 show 63 almost complete chromosomes, besides a number of ends. Undoubtedly we have in each case the $4x$ number, or 64 chromosomes.

The daughter nuclei.—During telophase the chromatin strands remain remarkably distinct (figs. 49–52). They elongate greatly, and become irregularly looped (figs. 50, 52). It would seem that each is in contact with the periphery somewhere throughout its length. Contraction is followed by an increase in the size of the nuclear space; the latter is accompanied by a vacuolization of the chromatin (fig. 55). The nuclear outline is still lobed, the lobes corresponding to the loops of chromatin. The nuclear membrane forms late. As the nucleus continues to enlarge, the chromatin becomes still more discontinuous, but the outline of the strands may still be readily traced.

It is to be noted that of all the material which was inclosed by the membrane of the egg nucleus, only the chromatin is included in the newly organized daughter nuclei. The large vacuolate darkly staining bodies, the filaments pervaded by granules, and the fibers are all excluded when the membranes inclose the daughter nuclei. We may conclude that these materials, although they may be found within the nuclear membrane, are not essentially nuclear and are not directly concerned in mitosis. They are, at most,

cytoplasmic inclusions within the nucleus. The chromatin is the fundamental nuclear substance.

Discussion

The ventral canal cell.—The general tendency among the Coniferales is toward the reduction of the ventral canal cell. In the Abietineae a cell is cut off by a cell wall; in the Taxineae and Cupressineae, as groups, a ventral nucleus is formed, but no cell is organized; while in *Torreya* (15) there is no ventral cell, the nucleus of the central cell becoming the egg nucleus. In *Abies* the nucleus of the ventral canal cell functions as an egg. In *Pinus Laricio* (3, p. 278), "while the ventral canal cell nucleus usually disappears soon after it is formed, in some cases it persists, and its nucleus becomes as large as that of the oosphere, passing through a similar developmental history. New support is thus given to the theory that the ventral canal cell is the homologue of the egg."

NICHOLS (21) describes (fig. 90) "two nuclei resulting from the division of the ventral canal cell nucleus" in *Juniperus*. The ventral canal cell "is fairly persistent in *Tsuga* (20). When division is complete, its nucleus is equal in size and similar in structure to the nucleus of the egg, and for some time shows the same stages of development."

The most extreme development recorded is in the case of *Thuja*, described by LAND (14). "A number of the writer's preparations of *Thuja* lead him to believe that both the ventral nucleus and the egg, in the same archegonium, may be fertilized. In fig. 17 the proembryo is well advanced, while the ventral nucleus has formed a group of four cells. Another preparation shows eight cells with indications that walls are soon to appear. The probability of such a fertilization is strengthened by finding occasionally in the same ovule embryos growing upward into the nucellus, as well as downward into the endosperm" (p. 224). These facts and those already described for *Abies* remove any doubt that the ventral canal cell is potentially an egg.

The cytoplasmic "mantle" about the egg nucleus is present in most species of Coniferales. NICHOLS (21) describes it in *Juniperus* as follows: "The mass of cytoplasm and starch derived from the

male cell gradually surrounds the conjugating nuclei so that there is never any possibility of mistaking the fusion nucleus for an unfertilized egg nucleus." And NORÉN (**22**) states: "Nachdem die Kopulation der Kerne erfilgt ist legt sich das Plasma der Spermazelle und deren Stärke wie ein Mantel um die beiden Kerne herum." The description is similar for *Taxodium* (**4**), *Torreya* (**15**), *Sequoia* (**16**), and *Thuja* (**14**). In *Ephedra* (**13**), a dense cytoplasmic mass develops about the egg nucleus and extends downward in the cytoplasm of the egg. In *Abies* a finely granular cytoplasmic layer develops about the egg nucleus during its movement toward the center of the egg (figs. 7, 14). This area extends along the path of the nucleus in the form of a short streamer. The male nucleus penetrates the mantle, but there is no appreciable addition to its mass by cytoplasm accompanying the same (fig. 19). At the telophase of the first division, the excess material from the egg cytoplasm is added to the "mantle" (figs. 49, 50, 55).

The darkly staining bodies described in *Abies* as globular or irregularly shaped and vacuolate, according to conditions, have been variously interpreted. NICHOLS (**21**) writes: "In the egg nucleus frequently the entire chromatin content of the nucleus seems to have resolved itself into nucleoli and pseudo-nucleoli. Yet even after a study of a large number of preparations one is unable to formulate any satisfactory conclusions as to the nature of these structures." Describing the egg nucleus of *Taxodium*, COKER (**4**) says: "In addition to the reticulum and plastin nucleoli there are also present numbers of chromatin nucleoli." And LAWSON for *Libocedrus* (**17a**) states that "It is impossible to distinguish the true chromatin from the nucleoli and other irregularly shaped bodies which stained like chromatin, and seemed to be closely associated with the latter." These bodies seem to resemble very much the karyosomes which SHARP (**24**) has described in the resting nuclei of *Vicia Faba*. "They appear in connection with the chromatic network and resemble the latter in staining quality. They seem to represent an elaboration product of a process actively going on during rest." A study of *Abies* has led to the conclusion that the darkly staining bodies are storage materials derived from the cytoplasm.

A cytological study of fertilization in conifers has been made for a number of species: CHAMBERLAIN, *Pinus Laricio* (3); BLACKMAN, *Pinus silvestris* (2); Miss FERGUSON, *Pinus* (6); MURRILL, *Tsuga canadensis* (20); NORÉN, *Juniperus* (22); and NICHOLS, *Juniperus* (21). "After the male pronucleus is within the oosphere nucleus the chromatin of the two pronuclei appears as two distinct masses in the spirem stage" (3). "Es scheint als würde jede der beiden Chromatingruppen zuerst ihre eigen Kernspandel ausbilden, die sich dann zu einzigen vereinigen" (22). On the fibers "the long bent and twisted chromosomes appear" in *Tsuga* (20). Miss FERGUSON (6) states: "When the chromosomes are being oriented at the nuclear plate, the maternal and paternal elements can no longer be distinguished." The number of chromosomes at this stage was found to be $2x$. No count is recorded during the phase of pairing (or splitting, as it has generally been regarded). Miss FERGUSON's figures confirm what has been described for *Abies*. Her figs. 236 and 237 may be compared with figs. 29–33; her fig. 238 with figs. 34–39; and her fig. 241 with fig. 51. As illustrated by diagram B, a number of stages in the process of fertilization have not been described heretofore. It is not surprising that, without these stages and not having the chromosome count throughout, the pairing should be interpreted as a longitudinal split, and that evidence of segmentation should not be found. Because of the complete series found in *Abies* and the extreme size of the chromosomes, it has been possible to discover the facts which center about the pairing, followed by the transverse segmentation.

Stages in fertilization.—A study of the union of egg and sperm in plants and animals makes it evident that there are several phases in the process of fertilization. The primary phase is illustrated by Uredineae. Here the fusion is evidently incomplete during the binucleate stages. "In the young aecidium the nuclei become paired and divide together in very close association. The teleuto-spores in the young state are binucleate, but when mature become uninucleate by the fusing of the two paired nuclei" (1). It is only after many separate but simultaneous divisions of the pairing nuclei that this second phase of the process is accomplished. HARPER (8) believes that bivalent chromosomes are formed in

Ascomycetes. "The time and degree of the combination of the sexual chromosomes is a variable matter. If the prochromosomes can remain in one nucleus with the double chromosome number, or in two distinct nuclei through part or all of the sporophyte generation, it is also possible that they may combine in one nucleus into bivalent chromosomes, and maintain their identity in this
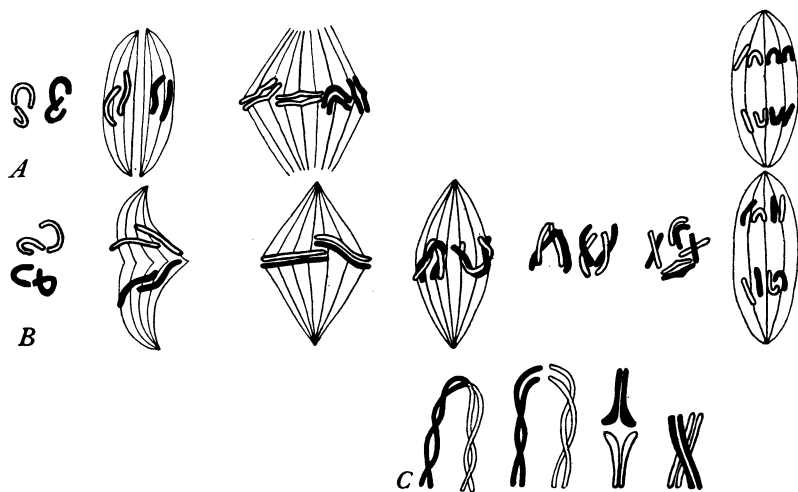

FIG. 1

Diagram *A*.—An interpretation of fertilization in some animals and in *Pinus*, according to the accounts heretofore recorded.

Diagram *B*.—An interpretation of the phenomena already described: the chromatin originating from the egg nucleus is shown in solid black, that from the male nucleus is outlined and barred; the argument upon which such an interpretation is based is included in the description given in the text; the facts of spindle formation are also indicated.

Diagram *C*.—A copy from GRÉGOIRE'S (7) "schéma de l'interprétation metasyndetique des tétrades-crois," as found in certain lower animals; compare fig. *B*.

condition through the sporophyte generation until a true reduction occurs in spore formation." In the well known case of *Cyclops*, studied by HAECKER, the parental chromosomes do not mingle, but persist as individuals and maintain their separation into two groups through several cell generations after "fertilization." We may consider the union of egg and sperm as consisting of three stages: (1) the two nuclei enter the same cytoplasmic mass; (2) the two

groups of chromosomes enter the same nuclear membrane, but retain their former grouping; and (3) the chromosomes pair. The first is illustrated by the paired nuclei in Uredineae; the second by the chromatin groups during the first divisions of the *Cyclops* embryo; the third takes place soon after the fusion of the egg and sperm in *Abies*. It seems altogether probable that the chromosomes do not actually pair until the prophase of the reduction division in such animals as *Cyclops*. There is a striking similarity between the pairing process as described in *Abies* and that concerned with tetrad formation in some animals (compare diagram *A* with diagram *C*). To what extent we are justified in suggesting that the processes are identical and that they are both concerned with the pairing of corresponding chromosomes from the egg and from the sperm, only further research can definitely determine.

Relationships

Abies, as compared with *Pinus*, shows a number of primitive characters:

1. The male gametophyte: (*a*) an excessive polar ("prothallial") tissue; (*b*) the equality of the male nuclei; both may function.

2. The female gametophyte: (*c*) the large number of neck cells; as many as five tiers (MIYAKI 19); (*d*) the persistence of the ventral canal cell; its nucleus may function as an egg nucleus.

3. The ovulate strobilus and ovules: (*e*) the almost complete separation of scale and bract; (*f*) the development of a rudimentary pollen chamber; (*g*) the comparatively free integuments.

4. The staminate strobilus: (*h*) the staminate strobili are borne on ordinary branches in the axes of ordinary leaves.

5. The arrangement of leaves: (*i*) the spiral arrangement of leaves on ordinary branches (THOMSON 25; LLOYD 18).

6. Vascular anatomy: (*j*) the general absence of ray tracheids (THOMPSON 26, 27), which in *Pinus* are present in the mature wood, but absent in the seedling and strobilus axis.

It may be noted, however, that resin canals are not found in the woody axis of the *Abies* stem except as traumatic responses (JEFFREY 11). JEFFREY states that the presence of resin ducts is an ancient character which has persisted in *Pinus*. "On account

of the reduced foliage of the abietineous conifers, this [that is, resinous secretion] was a very serious drain on the assimilatory apparatus. Gradually the more economical tendency arose of forming resin passages in the case of need only." So regarded, *Pinus* would be more primitive than *Abies*. Whether or not this argument is sufficient to overbalance the numerous ancient characters of *Abies* previously tabulated is a matter of judgment which we do not presume to decide.

Many of the foregoing characters are such that they tend to relate more closely the two ancient groups of Coniferales, the Abietineae and the Araucarineae.

Summary

1. *The male gametophyte.*—The polar ("prothallial") cells may divide mitotically. The body cell divides to form the male nuclei while within the spore coat. Under favorable conditions a "prothallial" cell may develop as an antheridial cell, a biantheridial gametophyte resulting. The male nuclei are equivalent; one fuses with the egg nucleus and frequently the other fuses with the ventral canal cell nucleus.

2. *The ventral canal cell and ventral proembryo.*—The ventral canal cell nucleus breaks through its wall into the egg cytoplasm, enlarges, and fuses with one of the male nuclei. A ventral proembryo is formed by two successive divisions.

3. *The egg nucleus.*—The egg nucleus enlarges to 60 times its original volume. At the time of fertilization (fig. 28) irregular, darkly staining, vacuolate masses, slender filaments pervaded by small granules, spindle fibers, and chromatin are differentiated within the nuclear membrane. The chromatin is the fundamental nuclear substance; the other bodies are accretions gained during the growth of the nucleus and excluded from the nuclei of the proembryo.

4. *Fertilization and the first division.*—After fusion two chromatin groups appear at the base of the egg nucleus; in each $2x$ chromosomes become separate; the two original spindles unite; the chromosomes approximate to form x pairs; they twist the one about the other and become looped; each of the components of a pair

segments medianly, that is, at the apex of the loop; $2x$ pairs of seg-
ments result; these separate to form $4x$ chromosomes; a new
spindle is formed and $2x$ chromosomes pass to each pole.

5. Fertilization may be regarded as having three phases: (1) sex
nuclei enter a common cytoplasm; (2) the two groups of chromo-
somes enter a common nuclear membrane; (3) the chromosomes
approximate in pairs. The first phase may be prolonged, as in
Uredineae; the second may be prolonged, as in some animals; or
the three phases may follow one another in rapid succession, as in
Abies. Attention is drawn to the similarity existing between the
phenomena connected with pairing in *Abies* and tetrad formation
in animals. It is suggested that they may be like processes occur-
ring at different stages of the life history.

I wish to express my thanks for many suggestions and helpful
criticisms given by Professor J. M. COULTER and Professor C. J.
CHAMBERLAIN, under whose direction the investigation was
pursued.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BLACKMAN, V. H., On fertilization, alternation of generations, and general
   cytology of the Uredineae. Ann. Botany **18**:323–326. 1904.
2. ———, On the cytological features of fertilization and related phenomena
   in *Pinus silvestris*. Phil. Trans. Roy. Soc. B **190**:395–426. 1898.
3. CHAMBERLAIN, C. J., Oogenesis in *Pinus Laricio*. BOT. GAZ. **27**:268–279.
   1899.
4. COKER, W. C., On the gametophyte and embryo of *Taxodium*. BOT. GAZ.
   36:1–27. *pls. 1–11.* 1903.
5. FERGUSON, MARGARET C., Contributions to the life history of *Pinus*, with
   special reference to sporogenesis, the development of the gametophyte, and
   fertilization. Proc. Wash. Acad. Sci. **6**:1–202. *pls. 1–24.* 1904.
6. ———, The developement of the egg and fertilization in *Pinus Strobus*.
   Ann. Botany **15**:435–479. 1901.
7. GRÉGOIRE, V., Les cinèses de maturation dans les deux règnes. La
   Cellule **26**:223–422. 1910.
8. HARPER, R. A., Sexual reproduction in the organization of the nucleus in
   certain mildews. Pub. Carnegie Inst. Wash. no. 37. 1–92. *pls. 1–7.* 1905.
9. HUTCHINSON, A. H., The male gametophyte of *Picea canadensis*. BOT.
   GAZ. **59**:287–300. *pls. 15–19. fig. 1.* 1915.

10. HUTCHINSON, A. H., The male gametophyte of *Abies balsamea*. BOT.
GAZ. 57:148–153. *figs. 15.* 1914.

11. JEFFREY, E. C., The comparative anatomy of the Coniferales and Abie-
tineae. Mem. Boston Soc. Nat. Hist. 1905.

12. ———, Comparative anatomy of Abietineae. Mem. Boston Soc. Nat.
Hist. no. 6. 1904.

13. LAND, W. J. G., Fertilization and embryogeny in *Ephedra trifurca*. BOT.
GAZ. 44:273–292. 1907.

14. ———, A morphological study of *Thuja*. BOT. GAZ. 34:249–259. *pls. 6–8.*
1902.

15. COULTER, J. M., and LAND, W. J. G., Gametophytes and embryo of
Torreya taxifolia. BOT. GAZ. 39:161–178. 1905.

16. LAWSON, A. A., On the gametophytes, fertilization, and embryo of *Sequoia
sempervirens*. Ann. Botany 18:417–444. 1904.

17. ———, The gametophytes, fertilization, and embryo of *Cephalotaxus
drupacea*. Ann. Botany 21:1–23. 1907.

17a.———, The gametophytes and embryo of the Cupressineae, with special
reference to *Libocedrus decurrens*. Ann. Botany 21:281–301. 1907.

18. LLOYD, F. E., Morphological instability, especially in *Pinus radiata*.
BOT. GAZ. 57:314–319. 1914.

19. MIYAKI, K., Contribution to the fertilization and embryogeny of *Abies
balsamea*. Bot. Centralbl. 14:134–145. 1903.

20. MURRILL, W. A., The development of the archegonium and fertilization
in the hemlock spruce, *Tsuga canadensis*. Ann. Botany 14:583–607.
pls. 31, 32. 1900.

21. NICHOLS, G. E., A morphological study of *Juniperus communis* var.
depressa. Bot. Centralbl. 25:201–241. 1910.

22. NORÉN, C. O., Zur Entwicklungsgeschichte des *Juniperus communis*.
Uppsala Univ. Arks. pp. 64. *pls. 4.* 1907.

23. OVERTON, J. B., On the organization of the nuclei in the pollen mother cells
of certain plants, with especial reference to the permanence of chromosomes.
Ann. Botany 23:19–60. 1909.

24. SHARP, L. W., Somatic chromosomes in *Vicia*. La Cellule 29:297–331.
pls. 2. 1913.

25. THOMSON, R. B., The spur shoot of pines. BOT. GAZ. 57:362–385. *pls.
20–23. figs. 2.* 1914.

26. THOMPSON, W. P., On the origin of ray tracheids in the Coniferae. BOT.
GAZ. 50:101–116. 1910.

27. ———, Ray tracheids in *Abies*. BOT. GAZ. 53:331–338. 1912.
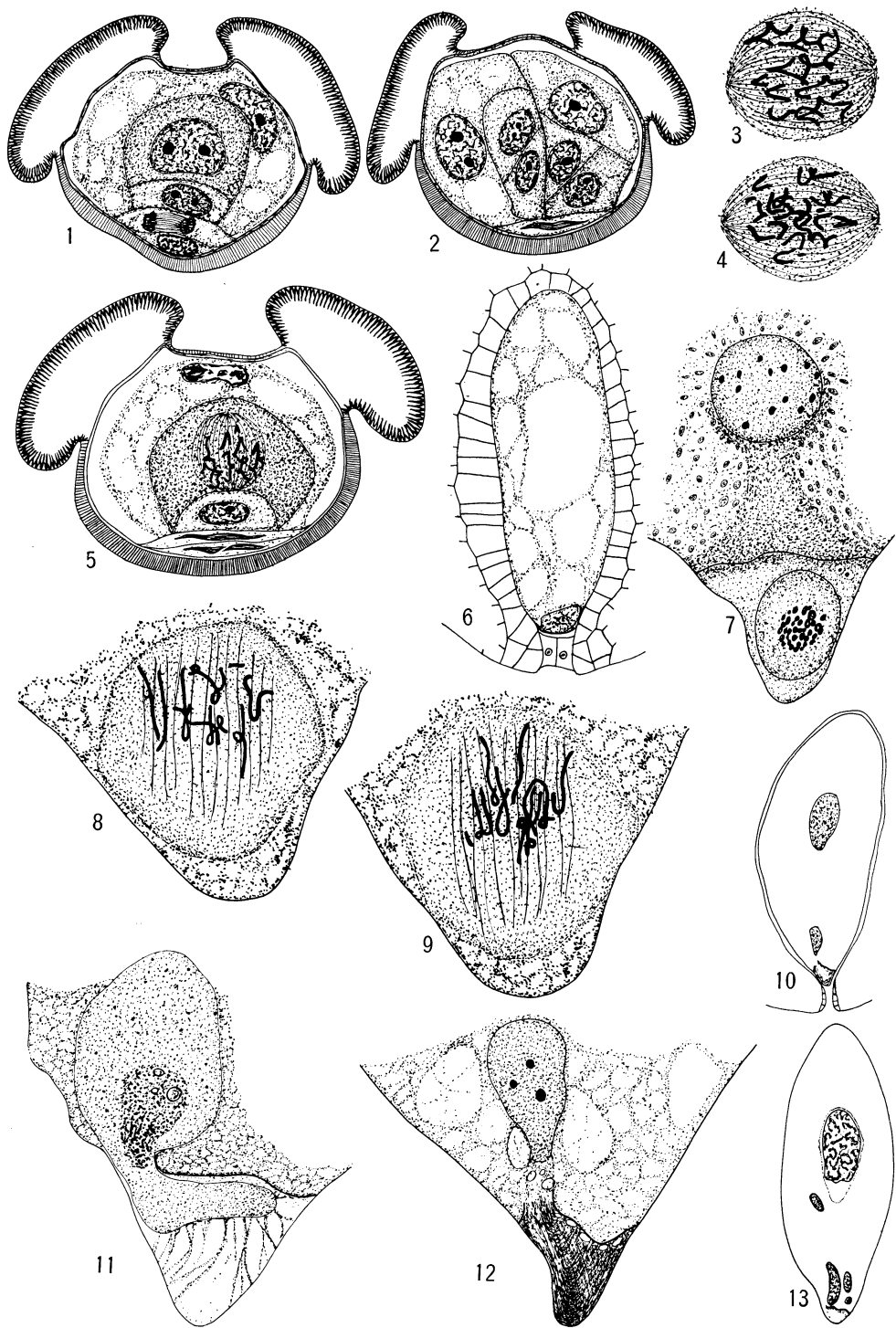
EXPLANATION OF PLATES XVI–XX

FIGS. 1–5.—Pollen grains: male gametophyte.
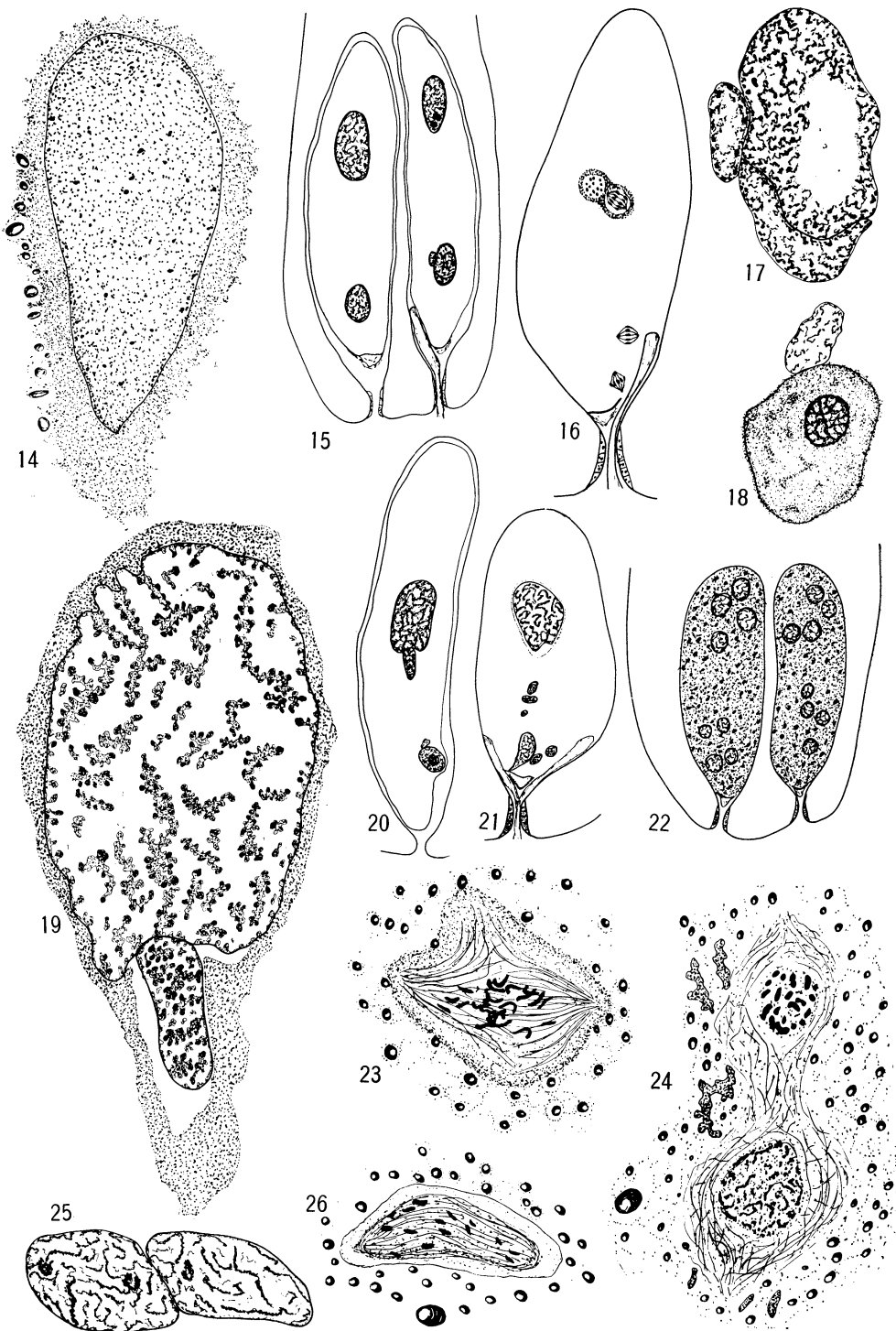
FIG. 1.—Division of polar cell; $\times 510$.
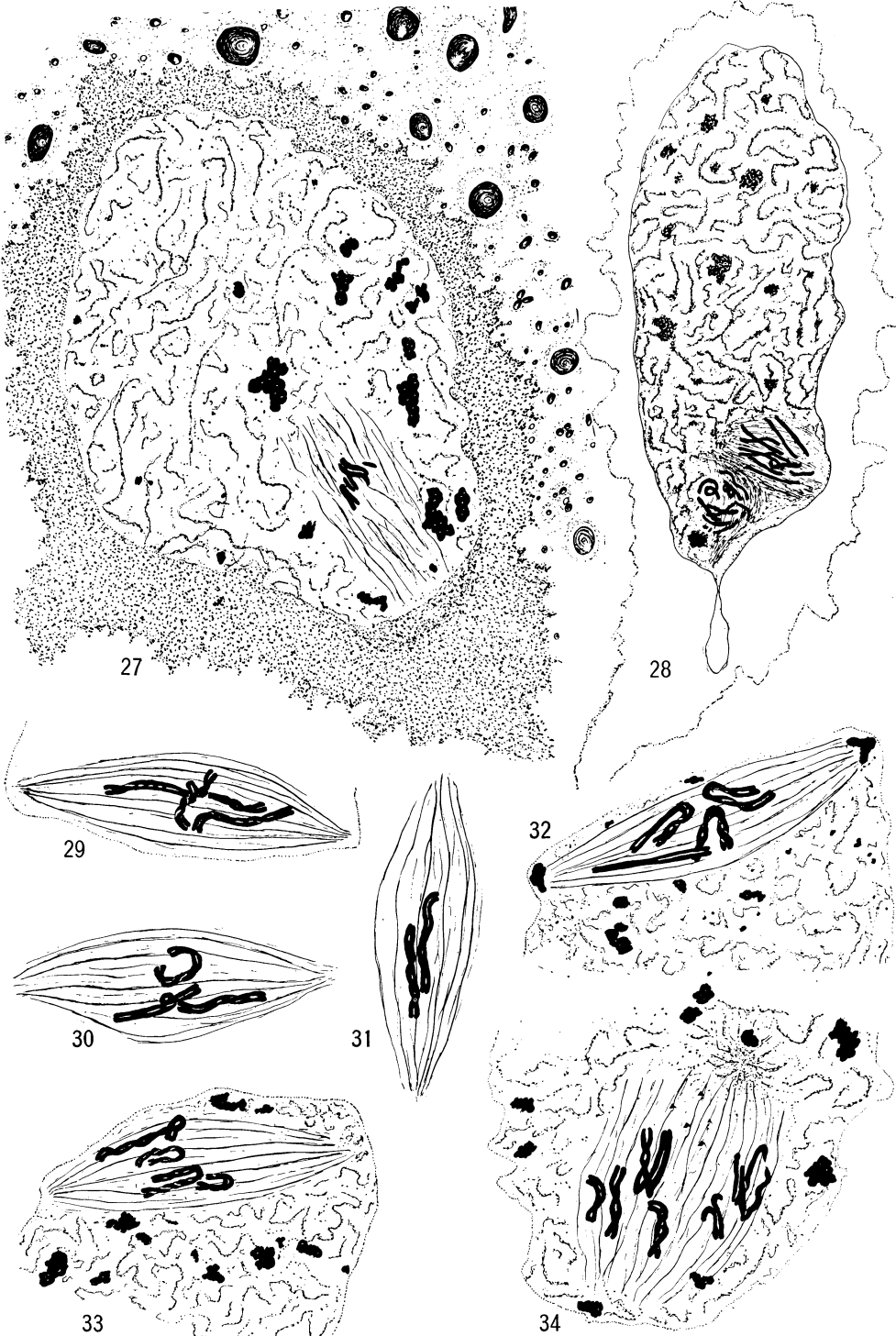
FIG. 2.—Biantheridial gametophyte; $\times 510$.

FIGS. 3–5.—Division of body cell; ×865.

FIG. 6.—Central cell and two neck cells; ×510.

FIG. 7.—Ventral canal cell and egg nucleus; ×510.

FIGS. 8, 9.—Mitosis of nucleus of central cell; ×865.

FIG. 10.—An archegonium: egg nucleus, ventral canal cell with wall broken and escaped nucleus in the cytoplasm of the egg; ×85.

FIGS. 11, 12.—Nucleus of ventral canal cell escaping.

FIG. 13.—Egg nucleus near a male nucleus; ventral canal cell nucleus, and near by the other male nucleus and stalk nucleus.

FIG. 14.—Detail of egg nucleus of fig. 10.

FIG. 15.—Two archegonia, one showing fertilization of both egg and ventral canal cell nucleus; ×85.

FIG. 16.—Divisions following the two fusions; ×85.

FIG. 17.—Ventral canal cell nucleus: a male nucleus and the stalk nucleus in contact; ×510.

FIG. 18.—Fusion nucleus resulting from union of ventral canal cell nucleus and male nucleus; ×510.

FIG. 19.—Egg nucleus and male nucleus in contact; ×510.

FIG. 20.—Egg and male nucleus in contact (see detail in fig. 19): the ventral canal cell nucleus as described for fig. 18; ×510.

FIG. 21.—An archegonium showing the egg nucleus, ventral canal cell nucleus, four male nuclei, a stalk nucleus, and two pollen tubes; ×510.

FIG. 22.—Postion and relation of ventral proembryo and proembryo proper; ×510.

FIGS. 23–26.—Detail of division following the fertilization of the ventral canal cell nucleus; ×865.

FIG. 27.—Nucleus of the fertilized egg; ×510.

FIG. 28.—Nucleus of the fertilized egg: two groups of chromosomes; ×510.

FIGS. 29–33.—Pairing of chromosomes; ×865.

FIGS. 34–40.—Segmentation of chromosomes; ×865.

FIGS. 41, 42.—Paired segments; ×865.

FIGS. 43–45.—Chromosome segments intermingling, still somewhat paired; ×865.

FIGS. 46–48.—Chromosomes starting to the poles; ×865.

FIG. 49.—Early telophase of first division; ×865.

FIGS. 50, 52, 55.—Late telophase of first division; ×865.

FIG. 51.—Anaphase of first division; ×865.

FIG. 53.—Resting stage of nucleus from ventral proembryo; ×865.

FIG. 54.—Resting stage of nucleus of the proembryo; ×865.

14

15

16

17

18

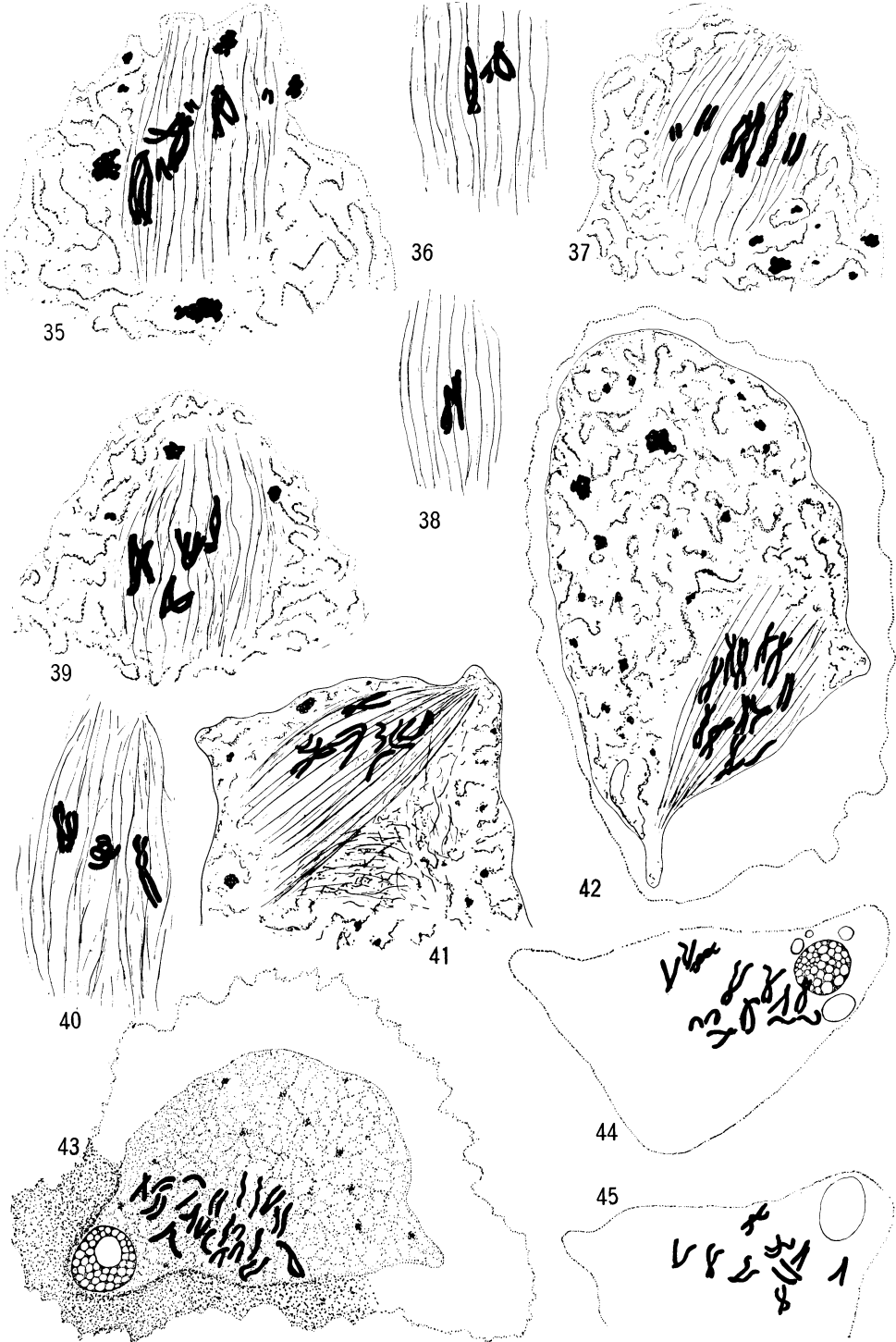19

20

21

22

23

24

25

26